A.K. KOUL* & A.K. WAKHLU*: Studies on the genus *Gagea* (5) Embryology of *Gagea reticulata* Schult**

A.K. コウル*・A.K. ワクル*: キバナノアマナ属の研究 (5) *Gagea reticulata* の胚学的研究

Species of *Gagea* (Liliaceae) have been investigated in the past by several embryologists (Nemec 1912, Stenar 1927, Romanov 1936, Joshi 1940, Koul et al. 1969, 1976, Gvaladze 1974, Saddiqi & Hashmi 1975). Barring a few papers, most of the published information concerns the ontogeny of embryo sac. Among the species that grow in Himalayas, *G. kashmiriensis* and *G. stipitata* alone have been studied for embryology; the rest, *G. reticulata, G. dshungarica, G. gageoides* and *G. elegans* are unexplored. The present communication describes the structure and development of anther and ovule and the pre- and post-fertilization events in *G. reticulata,* a diploid species. The development of gametophytes has been compared in order to get insight into the breeding system of this wild species.

Material and methods Material of *Gagea reticulata* was collected locally at regular intervals and fixed at the spot in freshly prepared formalin-acetic-alcohol (5:5:90). After routine dehydration and paraffin embedding, the material was sectioned between 8-16 μm. Safranin-fast green combination was used for purposes of staining.

Observations *G. reticulata* grows in gardens and on 'kerewas' in the association of lawn grass at altitudes varying from 400-1600 m. The plants prefer dry clayey soil and flower from March-April in Kashmir and January-February in Jammu.

Plants bear one angular radical leaf and 3-4 yellow stellate flowers (Fig. 1). The flower has six perianth lobes arranged in two whorls of three each. The lobes are acuminate, yellow from inside with a greenish tinge outside, 1-1.8 cm long, 0.2-0.35 cm broad. Ovary trilocular, broad above and tapering at the base. Placentation is axile and the seeds are triangular, flat and reddish.

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Structure of anther. Anther is four-lobed with the parietal tissue in each lobe consisting of epidermis, endothecium, middle layer and tapetum (Fig. 2A). The epidermal cells undergo radial enlargement and become vacuolate during advanced stages of anther development. All endothecial cells except those along the dehiscence slit develop fibrous thickenings on radial walls (Fig. 2F). This wall layer persists till the pollen grains differentiate within the microsporangium. The tapetal cells are isodiametric and densely cytoplasmic bearing a prominent nucleus. This layer is best developed at the time when sporogenous cells enter into meiosis. Although generally uninucleate, sometimes the tapetal cells become binucleate. The tapetum is of the glandular type. Remnants of tapetum keep scattered as deep staining streaks inside each locule (Fig. 2E).

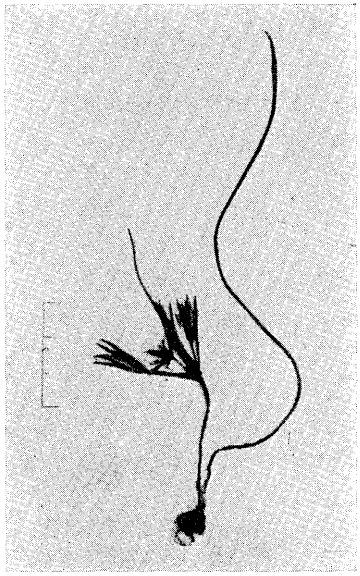

Fig. 1. Specimen of *G. reticulata* bearing a single angular radical leaf.

Initially, the sporogenous tissue is composed of compact polygonal cells. Before entering into meiosis, these cells round off and develop hyaline callose wall all round. Meiosis is regular. Wall formation is of the successive type (Fig. 2B). Microspore tetrads are mostly of the isobilateral type (Fig. 2C).

Uninucleate pollen grains are spherical. The first pollen mitosis takes place very near the wall. Cytokinesis results in the differentiation of a large vegetative and a small generative cell (Fig. 2D). In older pollen grains, generative cell separates out from the wall and rounds off. Mature pollen grains measure 39×31 μm. The haploid chromosome complement of the pollen grains conforms in details to the somatic complement. Occasionally pollen grains with deviant complement are observed. These pollen grains contain four long subterminal chromosomes against three within the standard karyotype (Fig. 3A, B). At the same time they have one median chromosome less.

Dimorphic pollen grains are produced by some plants of the natural popula-

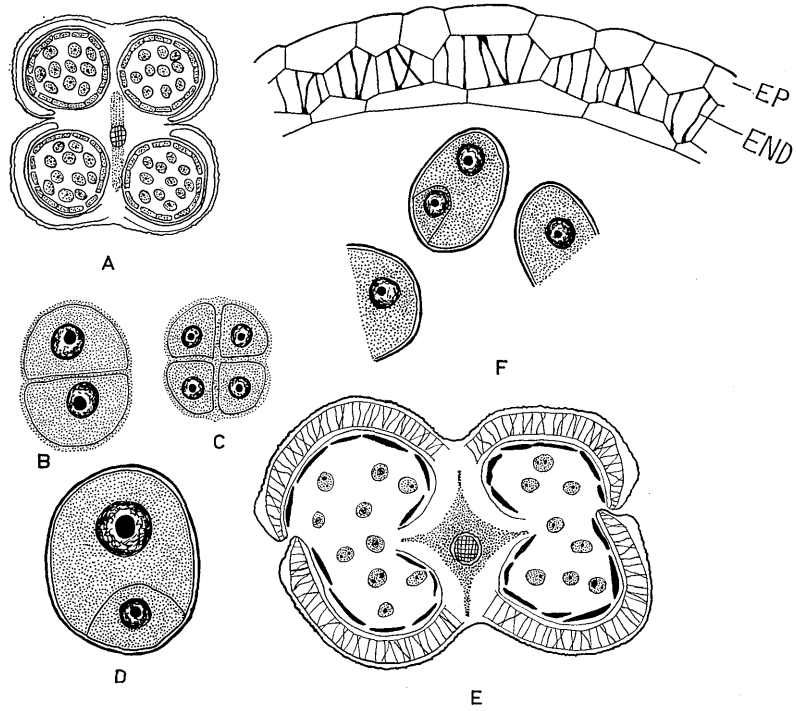

Fig. 2. Microsporangium, microsporogenesis and male gametophyte (EP, epidermis; END, endothecial thickenings). A: Transverse section of an anther, ×160. B-C: A dyad and microspore tetrad, ×1000. D: A bicelled pollen grain, ×1000. E: Transverse section of an anther showing the lateral dehiscence slits and degenerated tapetum, ×100. F: A portion of anther wall and the pollen grain, ×600. Note the fibrous thickenings of the endothecial cells.

tion. The large-sized pollen grains bear deep furrows on their surface (Fig. 3C-F). These appear to represent incompletely divided microspore mother cells.

While gametogenesis is heading completion, the adjacent lobes of the anther become confluent by the breaking down of partition walls. Once the pollen grains mature, the anther wall gives way along the lateral dehiscence slit (Fig. 2E).

Development of ovule. The ovule is anatropous, bitegmic and tenuinucellate. The integumentary initials appear at the megaspore mother cell stage (Fig. 4B). The initiation as well as development of the inner integument precedes that of the outer. The two-layered inner integument elongates and forms the promi-

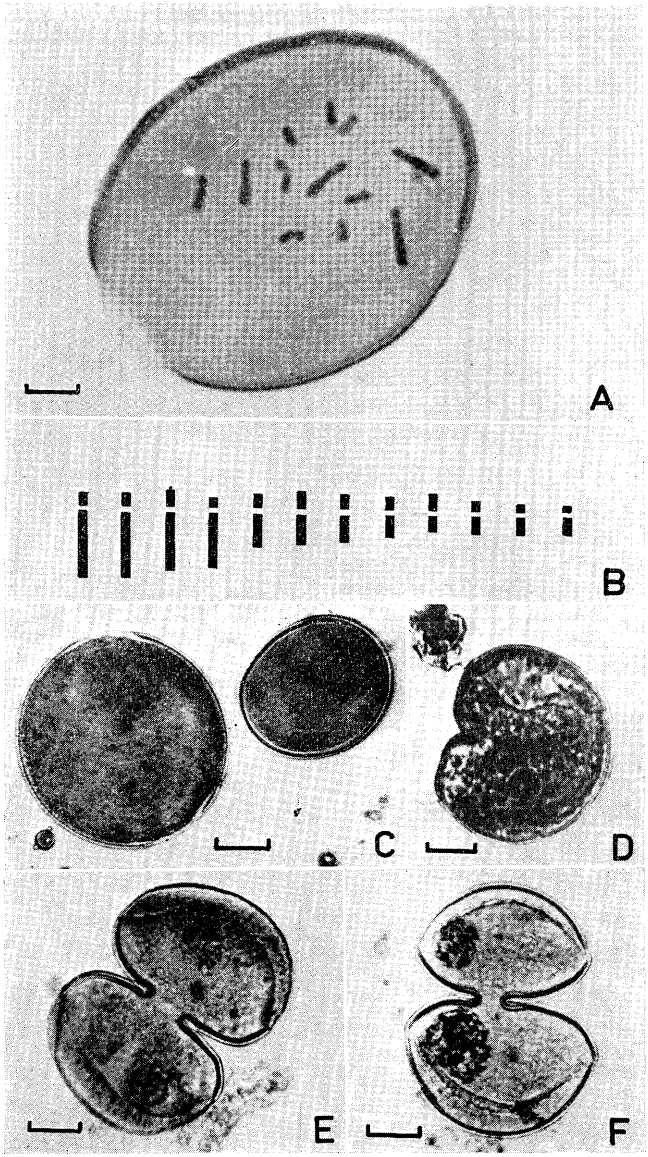

Fig. 3. Pollen grain divisions. A: Pollen mitosis showing structurally altered chromosome complement. B: Idiogram of the haploid complement in Fig. 3A. Note four long chromosomes instead of three present in normal haploid complement. C: Dimorphic pollen grains. Note difference in size. D: A pollen grain where furrowing has just started on one side. E-F: Pollen grains about to divide into halves. Scale A-B: 5 μ; C-F: 18 μ.

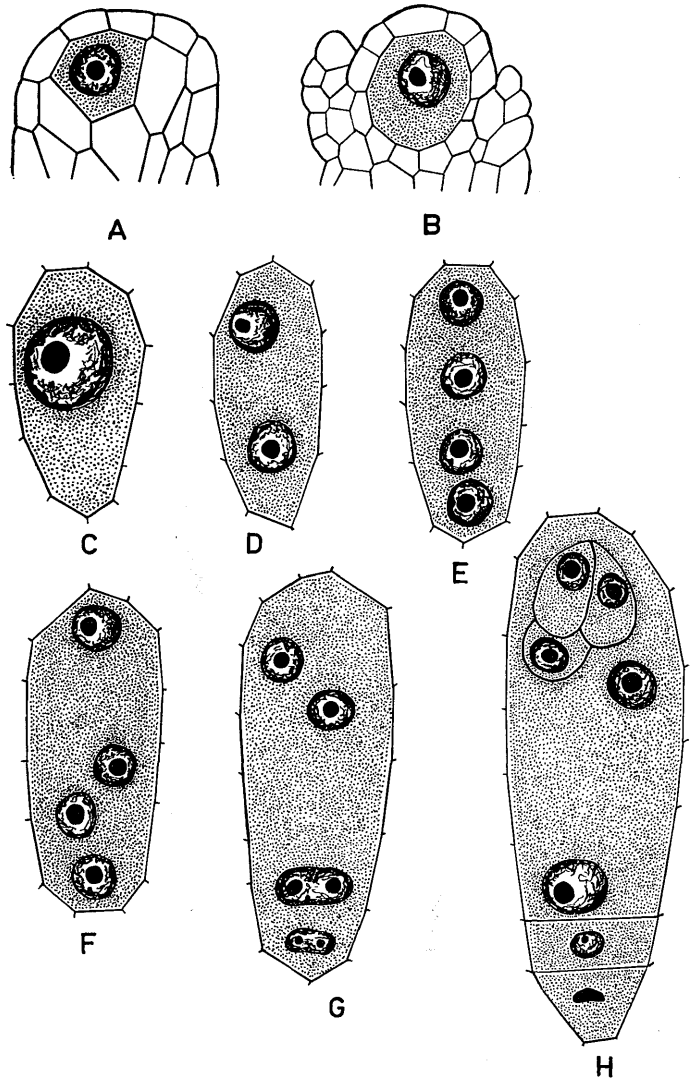

Fig. 4. Megasporogenesis and megagametogenesis. A-B: Longitudinal section of the ovule showing hypodermal archesporial cell and integumentary initials, ×600. C: A megaspore mother cell, ×600. D: A binucleate embryo sac, ×600. E: An embryo sac at primary 4-nucleate stage, ×600. F: An embryo sac showing 1+3 arrangement, ×600. G: An embryo sac at secondary 4-nucleate stage, ×600. H: An organized embryo sac, ×600.

nent micropylar canal. Cells at the tip of the inner integument enlarge considerably and become massive. Outer integument keeps smaller and does not contribute to the formation of micropyle.

Usually a single female archesporial cell, 16.5×14.2 μm in size, differentiates immediately beneath the nucellar epidermis (Fig. 4A). It enlarges and functions directly as the megaspore mother cell which is 26.4×19.8 μm in size (Fig. 4C). Female meiosis is regular and it results in the differentiation of two-nucleate embryo sac (Fig. 4D). Division of the two nuclei of the binucleate embryo sac results in formation of four nuclei which are arranged zig-zag or in a row; the arrangement depends on the breadth of the embryo sac (Fig. 4E). Average size of the primary 4-nucleate embryo sac is 66×33 μm. Subsequently three of the four nuclei migrate to the chalazal end; nuclear arrangement thereafter is 1+3 (Fig. 4F). Triple fusion leads to differentiation of the secondary 4-nucleate embryo sac, 82.5×33 μm in size (Fig. 4G). The nucleus present at the chalazal tip of the 4-nucleate embryo sac is smaller than the three above it. Unlike other three nuclei, the small nucleus does not undergo the third mitotic division with the result that a 7-nucleate embryo sac measuring 99×36.3 μm differentiates. The organized embryo sac has 3-celled egg apparatus, two polar nuclei and two antipodals (Fig. 4H). The basalmost antipodal cell degenerates precociously (Fig. 4H).

Pollination and fertilization. Anthers dehisce much after the flower opens. Pollen grains are monosiphonous. Fertilization is porogamous. In certain cases pollen tube persists within the micropylar canal and is visible as a deeply stained filament even at the time when the zygote enters into division. About 95% pollen is viable.

Embryo and endosperm. The primary endosperm nucleus undergoes numerous divisions to produce a mass of free nuclei (Fig. 5A). Obviously, the development of endosperm is of the nuclear type. Wall formation is initiated at the periphery of the embryo sac but ultimately the whole endosperm becomes cellular. Cells constituting the mature endosperm are polygonal, uninucleate and compactly packed (Fig. 5B).

The zygote enlarges considerably prior to division. A single transverse division divides it into two unequal cells (Fig. 5C). The basal cell does not divide but enlarges and becomes vacuolate and seems to function as a haustorium. The suspensor as well as embryo differentiate from the apical cell. The sus-

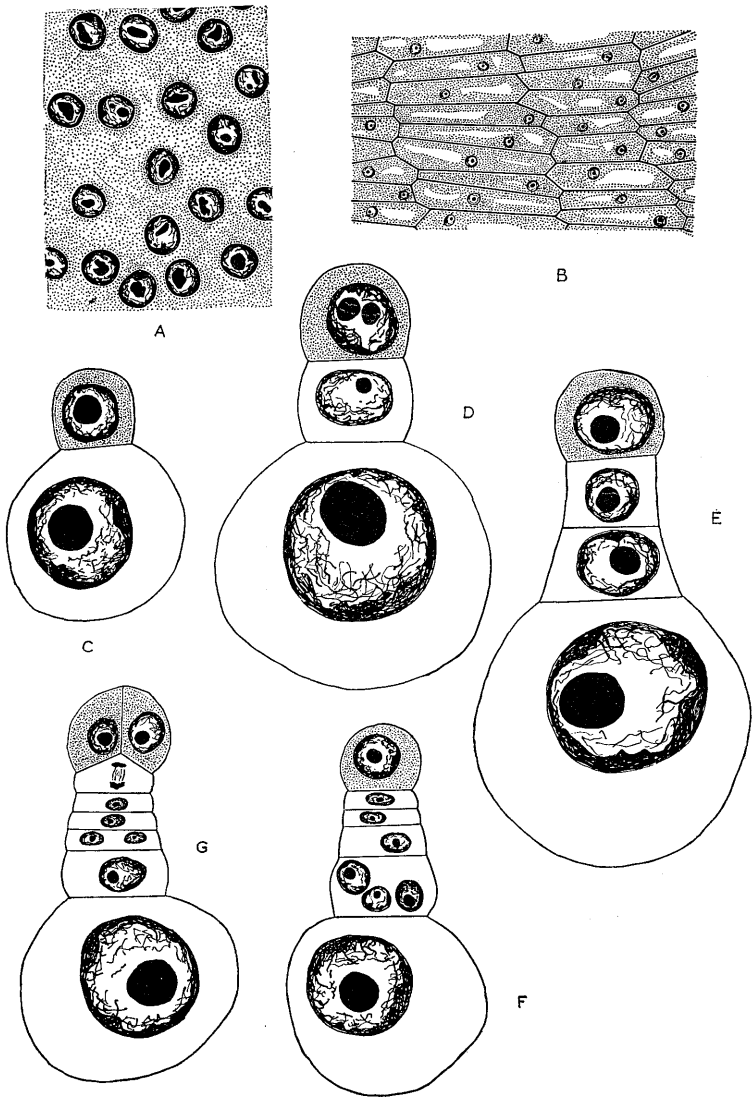

Fig. 5. Endosperm development and embryogenesis. A: A portion of free nuclear endosperm, ×450. B: A portion of cellular endosperm, ×450. C–G: 2-celled; 3-celled; 4-celled; 6-celled and 9-celled proembryo, ×1000. Note the undivided and inflated basal cell.

pensor is comprised of 6-9 linearly disposed cells located between the haustorium and the embryonal mass (Fig. 5D-G). As the embryo approaches maturity, the haustorium as well as suspensor degenerate.

Seed and seed coat. With enlargement of endosperm, the nucellus is totally consumed. Cells of the inner integument also get absorbed except in the micropylar region. Consequently, the outer integument comes in direct contact with the endosperm. Integumentary cells enlarge in size and develop thickenings to form the seed coat. The mature seed is 1.5 mm long. Seed set as well as fertility are quite high.

Discussion Of the two diploid gageas known from Kashmir, *G. kashmiriensis* has been studied for embryology by Koul, Wafai & Khan (1969). The course of events in *G. reticulata* corresponds those reported for *G. kashmiriensis*. Microsporogenesis and male gametogenesis lead to the differentiation of bicelled, spherical pollen grains. A few plants produced dimorphic pollen; some pollen grains being twice the size of standard ones. Similar variation in pollen size have been reported in *G. spathacea*, *G. bohemica*, *G. paczoskii* and *G. lutea* (Westergard 1936, Mesicek & Hrouda 1974). In these species size difference has been ascribed to erratic chromosome distribution at anaphase I and II. Anomalies in chromosome distribution and the ensuing difference in pollen size in *G. bohemica* and *G. lutea* is ascribed to unfavourable temperature (Mesicek & Hrouda 1974). The present findings indicate that the large-sized pollen grains represent microspore mother cells differentiated into monads without undergoing meiosis and the dyad-like structures are incompletely divided microspore mother cells which have become surrounded by exine. Similar structures observed in *Viola odorata* (Singh & Gupta 1963) and *Zea mays* (Koul 1969) have been interpreted as dividing pollen grains.

Ordinarily, the haploid and diploid chromosome complement of a species match in detail. In *G. reticulata* about 22.5% pollen grains produced by the plant carried structurally altered complements. The structurally altered chromosomes seem to represent products of unequal chromosome interchanges.

Like other species of the genus, embryo sac development in *G. reticulata* conforms to the '*Fritillaria* type'. The development of micro- and megaspore and male and female gametophytes do not synchronize in *G. reticulata*, precisely in the same way as in *G. kashmiriensis* and *G. stipitata* (Koul, et al. 1969, 1976). When meiosis in the male sporogenous track is completed, the arche-

sporium is yet to differentiate. Such a big time gap between the sequence of events in the two hereditary tracks leads to dichogamy. Protandry coupled with opening of flowers well before anthesis accounts for the outbreeding nature of this species.

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花粉の多くは花の裂開する前までに正常に発育して2細胞になる。ときに大きな花粉が混っているが，これは減数分裂を行なわなかったものと思われる。胚嚢形成は *Fritillaria* type で，大胞子母細胞は核分裂しても細胞分裂せず，4核がそのまま1個の胚嚢の形成に関係する。受精卵は上下に2分裂した後，下の細胞は大きな吸収器細胞となり，上の細胞によって胚形成が行なわれる。